

# Jak2 Is an Essential Tyrosine Kinase Involved in Pregnancy-Mediated Development of Mammary Secretory Epithelium

JONATHAN M. SHILLINGFORD, KEIKO MIYOSHI, GERTRAUD W. ROBINSON, SANDRA L. GRIMM, JEFFREY M. ROSEN, HANS NEUBAUER, KLAUS PFEFFER, AND LOTHAR HENNIGHAUSEN

*Laboratory of Genetics and Physiology (J.M.S., K.M., G.W.R., L.H.), National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892; Department of Molecular and Cellular Biology (S.L.G., J.M.R.), Baylor College of Medicine; Houston, Texas 77030; and Institute of Medical Microbiology (H.N., K.P.), Immunology, and Hygiene, Technical University of Munich, D-81675 Munich, Germany*

The PRL receptor (PrIR) and the signal transducer and activator of transcription 5a (Stat5a) are essential for the proliferation and differentiation of mammary epithelium during pregnancy. Based on tissue culture cell experiments, Jak2 is the tyrosine kinase responsible for the phosphorylation of both the PrIR and Stat5. We have now used a genetic approach to test the role of Jak2 in the mammary gland, a PrIR-responsive tissue. Because Jak2-null embryos die at E12.5, we transplanted Jak2-null mammary anlagen into cleared fat pads of wild-type mice and investigated epithelial development during pregnancy. In the absence of Jak2, no secretory alveoli were present at parturition, and ep-

ithelial cell proliferation was reduced by 95% after an acute hormone treatment. Furthermore, the Na-K-Cl cotransporter, a ductal marker, was maintained in Jak2-null epithelium and the sodium-phosphate cotransporter type IIb, a secretory cell marker, was absent. Nuclear Stat5a was only observed in a few epithelial cells in Jak2-null glands at pregnancy and parturition compared with most epithelial cells in wild-type glands. Taken together, our results demonstrate that Jak2 is a critical tyrosine kinase that conveys intracellular signals necessary for proliferation and differentiation of mammary epithelium during pregnancy. (*Molecular Endocrinology* 16: 563–570, 2002)

MAMMARY EPITHELIUM PROLIFERATES and differentiates during pregnancy through the combined action of growth factors and steroid hormones. In particular, the PrI-Stat5 pathway (where Stat5 refers to both Stat5a and Stat5b) plays a central role in alveolar development. Analyses of gene knockout mice have demonstrated that the PRL receptor (PrIR) (1) and the transcription factor, Stat5a (2), are required for functional lobuloalveolar development during pregnancy. Inactivation of the PrIR results in the inability of the alveolar compartment to proliferate and differentiate during pregnancy (1, 3, 4). Similarly, in the absence of Stat5a, alveoli fail to fully develop and differentiate (2). In contrast, inactivation of Stat5b does not significantly impair alveolar epithelial development during pregnancy (5), which can be attributed to the lower levels of Stat5b protein (6) and the ability of Stat5a to functionally compensate for the lack of Stat5b. However, we have recently demonstrated that both Stat5a and Stat5b are required for full development of the alveolar epithelium (7).

Strong Stat5 phosphorylation is detected in the mammary gland around midpregnancy and persists throughout lactation but is rapidly lost at involution (6). Because

the PrIR lacks intrinsic kinase activity (8), association with a kinase is required to elicit receptor activation and subsequent activation of Stat5. The PrIR activates Jak2 in mammary gland explants *in vitro* (9), and Jak2 is responsible for PrIR phosphorylation and subsequent Stat5 recruitment and activation in tissue culture cells (e.g. 10–13). However, a definitive *in vivo* role for Jak2 in the mammary gland has not been established.

Functional inactivation of the *Jak2* gene has determined its essential role in erythropoiesis in the liver during early embryonic development (14, 15). Because deletion of the *Jak2* gene results in embryonic lethality (E12.5), we have used mammary gland transplantation to study Jak2-null epithelial development in a wild-type host. Using this approach, we were able to address specifically whether Jak2 is required for functional development of the mammary epithelium during pregnancy, or if other kinases are capable of compensating for the loss of Jak2.

## RESULTS AND DISCUSSION

### Lack of Alveolar Development in the Absence of Jak2

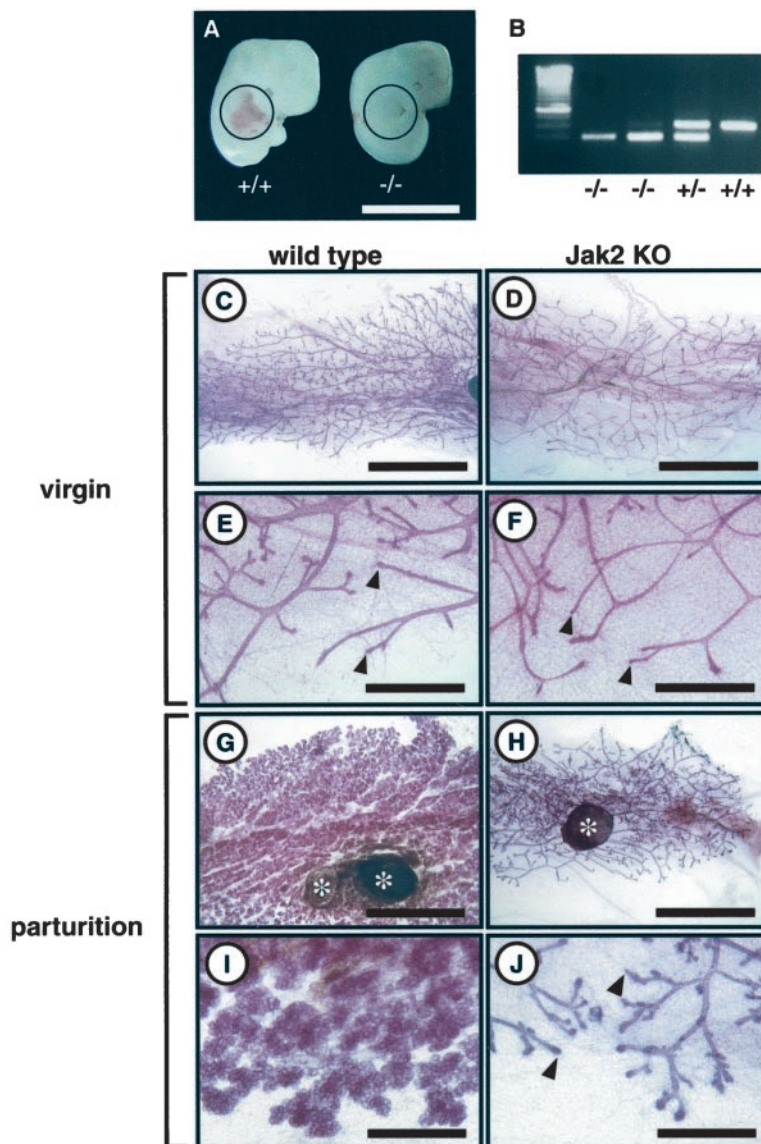
Inactivation of the *Jak2* gene leads to embryonic lethality around d 12.5 due to a lack of definitive eryth-

Abbreviations: BrdU, Bromodeoxy uridine; FITC, fluorescein isothiocyanate; NKCC1, Na-K-Cl cotransporter; Npt2b, sodium-phosphate cotransporter type IIb; PrIR, PRL receptor; Stat5a, activator of transcription 5a; TRITC, tetramethyl rhodamine isothiocyanate.

ropoiesis in the fetal liver (Fig. 1A). Therefore, to assess the role of *Jak2* in the development of the adult mammary gland, a transplantation approach was used. Thus embryonic mammary anlagen isolated from d 12.5 embryos (C57BL/6 genetic background) (Fig. 1, A and B) were transplanted into the epithelial-free mammary fat pads of athymic (nu/nu) nude mice, and mammary epithelial cell development was subsequently evaluated. Although athymic nude mice have reduced systemic levels of estrogen and progesterone

(16), the transplantation of wild-type and Jak2-null embryonic mammary glands within the same nude mouse allowed accurate comparisons to be made.

Whole mount analyses of virgin mice carrying wild-type (Fig. 1, C and E) and Jak2-null (Fig. 1, D and F) transplants revealed no significant differences. The Jak2-null epithelium completely filled the fat pad (Fig. 1D), and there was evidence of normal terminal ductal structures (see Fig. 1, E and F, *arrowheads*). In contrast, Jak2-null transplanted tissue harvested at par-



**Fig. 1.** Jak2 Is Essential for Alveolar Development in the Mammary Gland

A, Wild-type d 12.5 embryo (*left*) demonstrating erythropoiesis in the liver (*circle*). Note the absence of erythropoiesis in the liver (*circle*) of a Jak2-null embryo (*right*). B, PCR verification of embryonic *Jak2* genotypes. C–F, Development of ductal epithelium in virgin wild-type (C and E) and Jak2-null (D and F) transplanted hosts at 8 wk. Normal terminal ducts (E and F, *black arrowheads*) are apparent in both glands and no significant differences in overall development are observed. G–J, Alveolar development in wild-type (G and I) and Jak2-null (H and J) transplanted hosts at parturition. The epithelium completely occupies the fat pad in wild-type epithelium (G) and numerous alveolar structures are observed (I). No evidence of distinct alveolar structures are detected in Jak2-null epithelium (H) and the persistence of terminal duct structures is apparent (J, *black arrowheads*). The original embryonic transplants are depicted (G and H, *white asterisks*). A, Bar, 4 mm. C, D, G and H, Bar, 5 mm. E, F, I and J, Bar, 1 mm.

turition revealed a lack of identifiable alveolar structures (Fig. 1, H and J) compared with wild-type transplants (Fig. 1, G and I). The ductal tree was maintained similar to that observed in virgin glands (see Fig. 1, J and F) and terminal ductal structures were evident (Fig. 1J, *arrowheads*).

To further analyze the morphology, histological sections were prepared. Similar to the whole mount analyses, hematoxylin and eosin-stained sections demonstrated that the ducts in virgin wild-type transplants (Fig. 2A) and Jak2-null transplants (Fig. 2B) were comparable. At parturition, the wild-type transplants contained large alveoli (Fig. 2C) with expanded lumina (Fig. 2C, *asterisks*). The alveolar cells were actively secreting milk as judged from the presence of large lipid droplets in the cytoplasm (Fig. 2C, *arrowheads*). In contrast, there was no histological evidence of lipid droplets in the Jak2-null transplants (Fig. 2D), and a persistence of fat cells was apparent. Although alveoli-like structures were present (Fig. 2D, *arrow*), no secretory alveoli were observed. These data demonstrate that Jak2 is absolutely required for the proliferation and/or differentiation of mammary secretory cells during pregnancy.

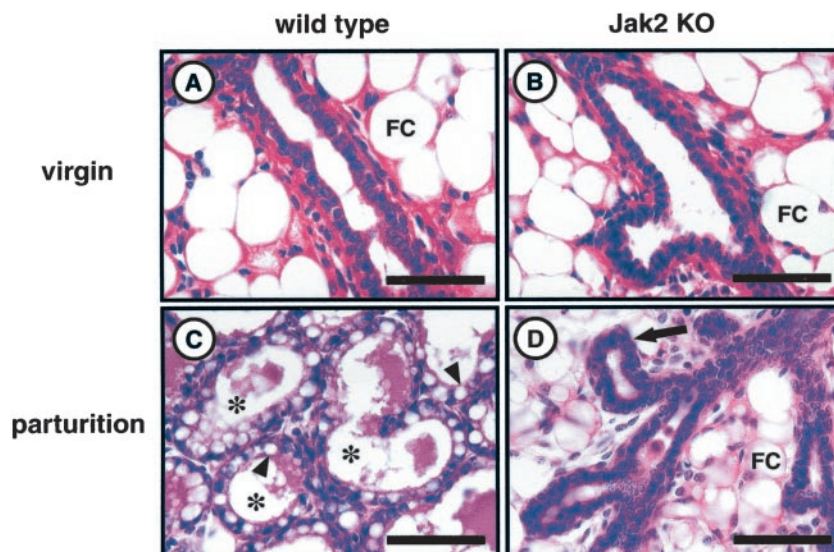
#### Reduced Proliferation in Jak2-Null Epithelium

To examine whether a decrease in proliferation was responsible for the lack of epithelium in Jak2-null transplants at parturition, the proliferative capacity of Jak2-null epithelium was assessed. Transplanted virgin mice (9-wk-old) were stimulated with estrogen and

progesterone for 48 h followed by bromodeoxy uridine (BrdU) injection. The incorporation of BrdU was subsequently examined using immunofluorescence.

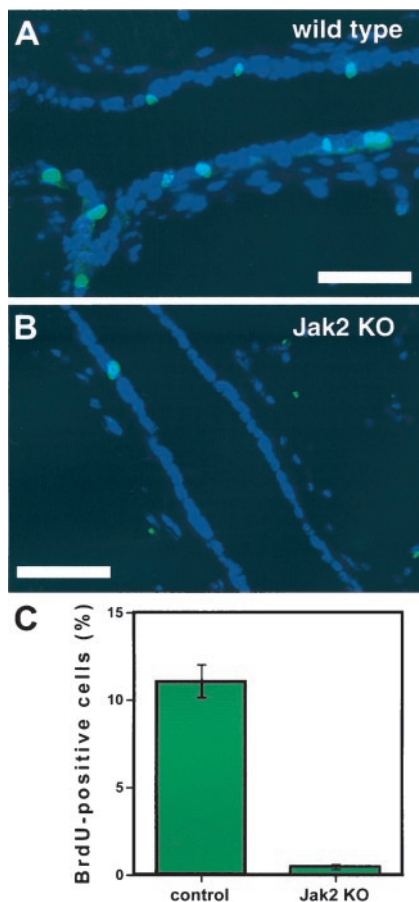
Remarkably, proliferation of Jak2-null ductal cells was reduced to 5% ( $P < 0.001$ ) of wild-type values (Fig. 3), indicating that Jak2 is required to elicit a hormonal-induced proliferative response in mammary epithelial cells. Furthermore, this demonstrates that proliferative responses in the mammary gland are largely mediated through Jak2-dependent mechanisms. Interestingly, proliferation in PrIR-null mammary cells was 30% of that observed in wild-type cells (7). This suggests that PrIR-independent signaling pathways mediate proliferative responses in the mammary gland. This could include signals emanating from the epidermal growth factor and GH receptors, which can activate Stat5 (4), and the glucocorticoid receptor, which can synergize with Stat5 (17). Indeed, recent data have demonstrated that GH, presumably via Jak2, can induce Stat5 phosphorylation in mammary stromal cells and epithelial cells (4). Although IGF-1 plays a significant role in mammary gland development (18) and is capable of activating Jak2 in NIH3T3 cells (19), it remains to be established whether Jak2 can mediate IGF-1 actions in the mammary gland.

Unfortunately, due to the fact that nuclear Stat5 protein is detectable in the Stat5-null mammary gland, consistent with the activation of a truncated Stat5 protein (unpublished data), we were unable to accurately compare proliferative responses in Jak2-null cells vs. Stat5-null cells.



**Fig. 2.** Jak2 Is Required for Functional Differentiation of the Mammary Epithelium

The whole mounts in Fig. 1 were embedded in paraffin, sectioned and processed for hematoxylin and eosin staining. No apparent differences in overall epithelial development are observed when comparing virgin wild-type (A) with Jak2-null (B) transplanted epithelium at 8 wk. Alveolar development in wild-type (C) and Jak2-null (D) transplanted epithelium at parturition. Alveoli with large, expanded lumen and luminal secretory products are evident in the wild-type epithelium (C, *asterisks*). Lipid droplets are also apparent in the secretory cells (C, *arrowheads*). No lipid synthesis is observed in Jak2-null epithelium (D), and the presence of a putative nonsecretory, alveoli-like structure is indicated (D, *arrow*). FC, Fat cell. Bar, 50  $\mu$ m.



**Fig. 3.** Reduced Proliferation of Epithelial cells in the Absence of Jak2

Transplanted mice were injected with estrogen and progesterone (E + P). After E + P treatment, mice were injected with BrdU and the glands harvested and subsequently fixed. Incorporation of BrdU was detected using a FITC-conjugated anti-BrdU antibody (green) and nuclei were visualized with DAPI (blue). BrdU localization in (A) wild-type and (B) Jak2-null epithelium showed far fewer BrdU-positive cells in Jak2-null epithelium. C, Counts revealed a 96% reduction in BrdU-positive cells in Jak2-null ( $0.47\% \pm 0.15$ ) vs. wild-type ( $11.26\% \pm 0.9$ ) epithelium (mean  $\pm$  SEM). It should be noted that the majority of ducts in the Jak2-null samples were BrdU-negative and the image shown in (B) depicts a rare proliferating cell. Bar, 50  $\mu$ M.

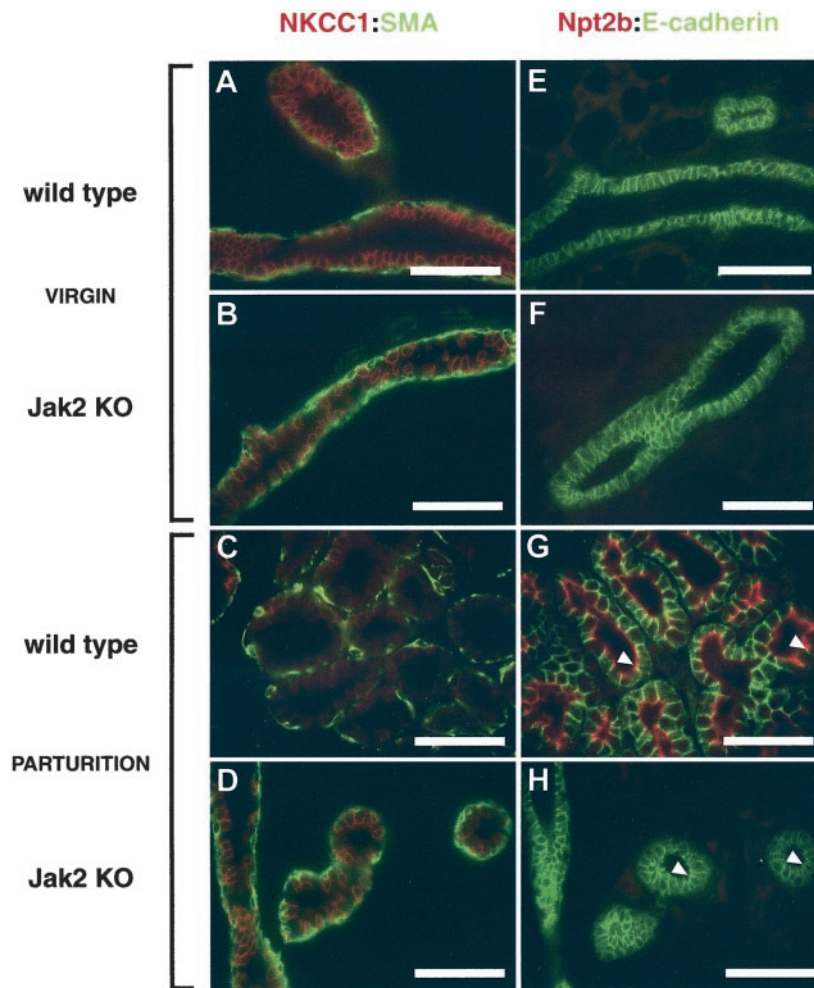
#### Maintenance of a Ductal Cell Marker and Lack of a Secretory Cell Marker in Jak2-Null Epithelium at Parturition

It was clear from the whole mount and histological analyses that the absence of Jak2 resulted in a failure of mammary epithelium to develop into distinct alveolar structures at parturition. Furthermore, Jak2-null transplants at pregnancy d 13 were also underdeveloped and were comparable to epithelial development seen in Jak2-null transplants at parturition (data not shown).

To investigate whether Jak2-null epithelium at parturition retained ductal characteristics and failed to acquire features characteristic of secretory epithelium, we investigated the expression of markers preferentially expressed in ductal or secretory epithelial cells. We have established that the Na-K-Cl cotransporter, NKCC1, is present at high levels on the basolateral membrane of ductal epithelial cells during virgin mammary gland development (Fig. 4A; and Shillingford, J. M., K. Miyashi, M. Flagella, G. E. Shull, and L. Henninghausen, unpublished data). During pregnancy (data not shown) and lactation (Fig. 4C) the levels of NKCC1 in wild-type alveoli are much reduced and only a few cells within the ducts maintain high levels of NKCC1 (data not shown). By EST database searches we also determined that an epithelial Na-Pi cotransporter, Npt2b (20), was preferentially expressed in cDNA libraries derived from lactating but not from nonlactating mammary tissue (data not shown). Therefore, these data suggest that NKCC1 and Npt2b serve as markers for ductal and secretory epithelial cells, respectively.

Jak2-null epithelium isolated from virgin mice (Fig. 4B) showed equivalent levels of NKCC1 protein as wild-type virgins (Fig. 4A). However, while a reduction in NKCC1 protein was observed in wild-type epithelium at pregnancy (data not shown) and parturition (Fig. 4C), Jak2-null epithelium maintained high levels of NKCC1 protein at parturition (Fig. 4D) similar to wild-type virgin epithelium (Fig. 4A). A comparable maintenance of NKCC1 expression was observed in transplanted Stat5- and PrIR-null epithelium at parturition (7). Taken together, the observed persistence of NKCC1 protein in Jak2-null epithelium at parturition demonstrates that the absence of Jak2 results in a lack of differentiation of the mammary epithelium during pregnancy and the retention of ductal features.

We further examined the expression of Npt2b. In wild-type glands, Npt2b protein was not detectable in ductal epithelium in virgin tissue (Fig. 4E) or alveolar structures at midpregnancy (data not shown). However, at late pregnancy (d 18) Npt2b was detected on the apical membrane of a few secretory alveoli (data not shown) and on the apical membrane of all alveoli at parturition (Fig. 4G, arrowheads). These results suggest that Npt2b is a marker of secretory cell function. Similar to virgin wild-type epithelium, ductal epithelium in Jak2-null virgin transplants showed no evidence of apical Npt2b (Fig. 4F). However, in contrast to wild-type epithelium at parturition, apical Npt2b was not detectable in Jak2-null epithelium at parturition (Fig. 4H). Based on maintenance of a high level of basolateral NKCC1 protein and lack of apical Npt2b protein, we conclude that Jak2-null mammary epithelium retains ductal characteristics and fails to acquire a marker indicative of secretory function.



**Fig. 4.** Maintenance of a Ductal Cell Marker and Lack of a Secretory Cell Marker in Jak2-Null Epithelium

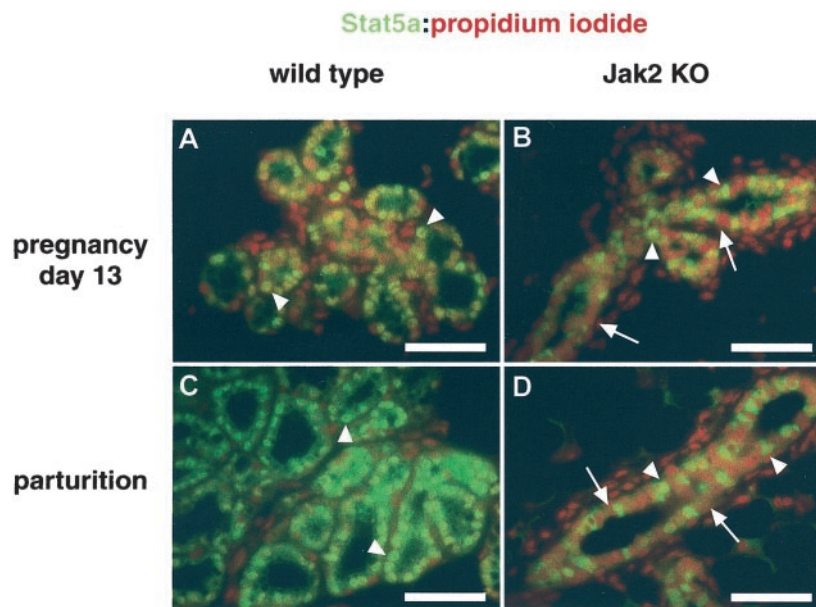
Transplanted tissue was harvested from virgin mice and mice 1 d after parturition. After fixation, tissue was embedded in paraffin, sectioned, and subsequently stained with specific antibodies. Localization of NKCC1 (red) and smooth muscle actin (SMA, green) in (A) virgin wild-type ductal epithelium, (B) Jak2-null virgin epithelium, (C) wild-type epithelium at parturition, and (D) Jak2-null epithelium at parturition. Note the persistence of a high level of NKCC1 protein in Jak2-null epithelium at parturition (D) similar to virgin wild-type ductal epithelium (A). In contrast, much reduced levels of NKCC1 are observed in wild-type alveoli at parturition (C). Localization of Npt2b (red) and E-cadherin (green) in (E) virgin wild-type ductal epithelium, (F) virgin Jak2-null epithelium, (G) wild-type epithelium at parturition and (H) Jak2-null epithelium at parturition. Npt2b protein is evident on the apical membrane of wild-type alveoli at parturition (G, arrowheads) but is notably absent in Jak2-null epithelium at parturition (H, arrowheads). No detectable Npt2b is apparent in virgin wild-type (E) or Jak2-null epithelium (F). Bar, 50  $\mu$ m.

#### Nuclear Translocation of Stat5a in the Absence of Jak2

It is well established that phosphorylation of Stat5 induces Stat5 dimerization, which in turn results in subsequent Stat5 nuclear translocation (21). In the context of the mammary gland PRL activates Stat5 via the PrIR and its associated kinase, Jak2, and the absence of either the PrIR or Stat5 inhibits functional differentiation of the mammary epithelium at pregnancy (7). Therefore, we examined the localization of Stat5a in wild-type and Jak2-null epithelium isolated from mice at pregnancy d 13 (Fig. 5A and B, respectively) and at parturition (Fig. 5, C and D, respectively). In wild-type glands at pregnancy d 13 (Fig. 5A), nu-

clear Stat5a staining was observed (arrowheads). In contrast, many fewer cells exhibited nuclear Stat5a in Jak2-null epithelium (Fig. 5B, arrowheads), and a large number of cells had no detectable Stat5a (arrows). After parturition, nuclear Stat5a was apparent in almost every epithelial cell in wild-type glands (Fig. 5C, arrowheads). Similar to that seen in pregnancy, Jak2-null epithelium at parturition (Fig. 5D) showed evidence of a few cells with nuclear Stat5a (arrowheads), cells with cytoplasmic Stat5a, and cells with no detectable Stat5a (arrows).

To quantitate Stat5a nuclear localization, the number of Stat5a-positive nuclei were counted and expressed as a percentage of the total number of luminal epithelial cells (Table 1). Counting of two independent



**Fig. 5.** Nuclear Localization of Stat5a in the Absence of Jak2

Wild-type (A and C) and Jak2-null (B and D) mammary transplants were harvested at pregnancy d 13 (A and B) and at parturition (C and D). Nuclei were stained with propidium iodide (red). Significant nuclear Stat5a (green) staining was apparent in wild-type glands at pregnancy d 13 (A, arrowheads), but less so in Jak2-null epithelium (B, arrowheads), which contained a proportion of cells with no detectable Stat5a (B, arrows). At parturition, most of the wild-type alveoli contained nuclear Stat5a (C, arrowheads) whereas only a few Jak2-null cells showed evidence of Stat5a nuclear staining (D, arrowheads). Note that some cells lacked detectable Stat5a staining (D, arrows). Bar, 50  $\mu$ m.

**Table 1.** Nuclear Stat5a vs. Total Number of Luminal Epithelial Cells

	Jak2-null	Wild-type
Pregnancy d 13 A	26.8 $\pm$ 2.1%	71.2 $\pm$ 1.5%
Pregnancy d 13 B	23.1 $\pm$ 1.1%	74.8 $\pm$ 2.8%
Parturition A	19.6 $\pm$ 1.4%	79.7 $\pm$ 2.1%
Parturition B	25.7 $\pm$ 2.0%	79.9 $\pm$ 2.2%

samples revealed a highly significant difference ( $P < 0.0001$ ) between Stat5a-positive nuclei in Jak2-null cells vs. wild-type cells at pregnancy d 13 (25% vs. 73%, respectively) and at parturition (23% vs. 80%, respectively). The observed nuclear localization of Stat5a in the absence of Jak2 could be mediated by a number of alternative signaling pathways independent of Jak2 function, including activation of phosphatidylinositol-3-kinase (22, 23), c-Src (24, 25), MAPK (10, 26) and signaling pathways activated by the intrinsic kinase activity of the epidermal growth factor receptor (4). However, despite evidence of limited Stat5a nuclear translocation in Jak2-null epithelium, these cells fail to proliferate and differentiate to form functional secretory tissue. A possible reason for this is that a threshold of activated Stat5 may be required to enable cells to switch from a ductal cell lineage to an alveolar cell lineage, which is not achieved in the absence of Jak2. Such a threshold could be perceived as 1) the number of cells expressing nuclear Stat5 and/or 2) the absolute amount of Stat5a in the nucleus. Unfortun-

nately, an accurate quantitation of the absolute amount of nuclear Stat5, which would further address this important question, is not readily attainable. Despite this, the data presented herein provide strong experimental evidence that in the absence of Jak2, other signaling pathways do not play a major role in the proliferation or differentiation of the mammary gland. This is the first *in vivo* study that defines Jak2 as a critical mediator of proliferative and differentiation events in the mammary epithelium at pregnancy.

## MATERIALS AND METHODS

### Antibodies

The NKCC1 and Npt2b antibodies were gifts from Dr. Jim Turner, NIDCR, NIH (Bethesda, MD) and Dr. Jurg Biber, University of Zurich (Zurich, Switzerland), respectively. The E-cadherin and Stat5a (L-20) antibodies were obtained from Transduction Laboratories (Lexington, KY) and Santa Cruz Biotechnology, Inc. (Santa Cruz, CA), respectively.

### Experimental Animals

All animals used in the course of this study were treated within published guidelines of humane animal care.

### Mammary Gland Transplantation

This technique has been described previously (27). Briefly, Jak2 hemizygous mice in a pure C57BL/6 genetic back-

ground (14) were mated and euthanized at d 12.5 of pregnancy. Embryos were removed and identified visually for the loss of *Jak2*, as assessed by the lack of erythropoiesis in the liver, and confirmed by PCR as previously described (14). Embryonic mammary glands were dissected from the *Jak2*-null, hemizygous and wild-type embryos and cultured overnight. The endogenous mammary epithelium from the no. 4 gland of 3-wk-old athymic (*nu/nu*) mice was removed, and the embryonic mammary cultures were placed into the remaining epithelial-free fat pad. Routinely, *Jak2*-null and *Jak2* wild-type glands were contralaterally transplanted in the same mouse. Mice were left for 8 wk before isolation of virgin mammary tissue, or mating and mammary tissue harvest during pregnancy or at parturition. For some experiments, successful outgrowths were transplanted a second time into nude mice as previously described (28). Whole mount analyses were performed as described previously (29).

### Immunofluorescence Analyses

Isolated tissue was fixed in Telleyesniczky's fixative for 4 h at room temperature and embedded in paraffin by routine methods. Sections were boiled in an antigen unmasking solution (Vector Laboratories, Inc., Burlingame, CA) for 2 min followed by 10 min incubation. Primary antibodies were applied (NKCC1, 1:1000; Npt2b, 1:100; E-cadherin, 1:200; Stat5a 1:200) and the sections incubated for 1 h at 37°C (NKCC1, Npt2b and E-cadherin) or overnight at room temperature (Stat5a). Sections were incubated with fluorescent-conjugated secondary antibodies (Molecular Probes, Inc., Eugene, OR) for 1 h at room temperature in the dark. Mounting medium (Vectashield, Vector Laboratories, Inc.) was applied, and the sections were analyzed. Immunofluorescence was viewed under a Carl Zeiss Axioscop (Carl Zeiss, Inc., Thornwood, NY) equipped with filters for FITC (fluorescein isothiocyanate), TRITC (tetramethyl rhodamine isothiocyanate) and FITC:TRITC. For Stat5a nuclear counts, the total numbers of luminal epithelial cells vs. nuclear Stat5a positive cells were counted in ten separate fields at 63× magnification (63× objective plus 10× eyepiece = 630×) from two independent mice. Statistical significance was assessed using *t* test.

### Cell Proliferation Analyses

For proliferation experiments, virgin mice 9 wk after transplantation were treated for 48 h with 1  $\mu$ g  $\beta$ -E2 (Sigma, St. Louis, MO) and 1 mg progesterone (Sigma) in 100  $\mu$ l sesame oil via a single interscapular sc injection. Two hours before they were killed, mice were injected with 0.3 mg BrdU per 10 g body weight (Amersham Pharmacia Biotech, Arlington Heights, IL), and both of the transplanted number 4 inguinal mammary glands and an endogenous number 3 gland (control) were removed. Isolated tissue was fixed in 4% paraformaldehyde in PBS for 2 h at 4°C. BrdU-positive cells were detected by immunofluorescence as described previously (30). BrdU-positive cells and total nuclei were counted from 16 fields at 60× magnification (60× objective plus 10× eyepiece = 600×). Statistical analysis was performed using a two-tailed *t* test.

### Acknowledgments

Received October 24, 2001. Accepted November 30, 2001.

Address all correspondence and requests for reprints to: Dr. Jonathan Shillingford, Laboratory of Genetics and Physiology, National Institutes of Health, 9000 Rockville Pike, Room 105, Building 8, Bethesda, Maryland 20817. E-mail: jonshi@helix.nih.gov.

J.S. is supported by a DoD fellowship, No. DAMD17-00-1-0246.

### REFERENCES

- Ormandy CJ, Binart N, Kelly PA 1997 Mammary gland development in prolactin receptor knockout mice. *J Mammary Gland Biol Neoplasia* 2:355–364
- Liu X, Robinson GW, Wagner KU, Garrett L, Wynshaw-Boris A, Hennighausen L 1997 Stat5a is mandatory for adult mammary gland development and lactogenesis. *Genes Dev* 11:179–186
- Brisken C, Kaur S, Chavarria TE, Binart N, Sutherland RL, Weinberg RA, Kelly PA, Ormandy CJ 1999 Prolactin controls mammary gland development via direct and indirect mechanisms. *Dev Biol* 210:96–106
- Gallego MI, Binart N, Robinson GW, Okagaki R, Coschigano KT, Perry J, Kopchick JJ, Oka T, Kelly PA, Hennighausen L 2001 Prolactin, growth hormone, and epidermal growth factor activate Stat5 in different compartments of mammary tissue and exert different and overlapping developmental effects. *Dev Biol* 229:163–175
- Teglund S, McKay C, Schuetz E, van Deursen JM, Stravopodis D, Wang D, Brown M, Bodner S, Grosveld G, Ihle JN 1998 Stat5a and Stat5b proteins have essential and nonessential, or redundant, roles in cytokine responses. *Cell* 93:841–850
- Liu X, Robinson GW, Hennighausen L 1996 Activation of Stat5a and Stat5b by tyrosine phosphorylation is tightly linked to mammary gland differentiation. *Mol Endocrinol* 10:1496–1506
- Miyoshi K, Shillingford JM, Smith GH, Grimm SL, Wagner K-U, Takami O, Rosen JM, Robinson GW, Hennighausen L 2001 Signal Transducer and Activator Transcription 5 (Stat5) controls the proliferation and differentiation of mammary alveolar epithelium. *J Cell Biol* 155:531–542
- Nagano M, Kelly PA 1992 Absence of a putative ATP/GTP binding site in the rat prolactin receptor. *Biochem Biophys Res Commun* 183:610–618
- Campbell GS, Argetsinger LS, Ihle JN, Kelly PA, Rillema JA, Carter-Su C 1994 Activation of JAK2 tyrosine kinase by prolactin receptors in Nb2 cells and mouse mammary gland explants. *Proc Natl Acad Sci USA* 91:5232–5236
- Das R, Vonderhaar BK 1996 Involvement of SHC, GRB2, SOS and RAS in prolactin signal transduction in mammary epithelial cells. *Oncogene* 13:1139–1145
- Ali S 1998 Prolactin receptor regulates Stat5 tyrosine phosphorylation and nuclear translocation by two separate pathways. *J Biol Chem* 273:7709–7716
- Aoki N, Matsuda T 2000 A cytosolic protein-tyrosine phosphatase PTP1B specifically dephosphorylates and deactivates prolactin-activated STAT5a and STAT5b. *J Biol Chem* 275:39718–39726
- Selvaraj NG, Omi E, Gibori G, Rao MC 2000 Janus kinase 2 (JAK2) regulates prolactin-mediated chloride transport in mouse mammary epithelial cells through tyrosine phosphorylation of Na<sup>+</sup>-K<sup>+</sup>-2Cl<sup>-</sup> cotransporter. *Mol Endocrinol* 14:2054–2065
- Neubauer H, Cumano A, Muller M, Wu H, Huffstadt U, Pfeffer K 1998 Jak2 deficiency defines an essential developmental checkpoint in definitive hematopoiesis. *Cell* 93:397–409
- Parganas E, Wang D, Stravopodis D, Topham DJ, Marine JC, Teglund S, Vanin EF, Bodner S, Colamonici OR, van Deursen JM, Grosveld G, Ihle JN 1998 Jak2 is essential for signaling through a variety of cytokine receptors. *Cell* 93:385–395
- Kopf-Maier P, Mboneko VF 1990 Anomalies in the hormonal status of athymic nude mice. *J Cancer Res Clin Oncol* 116:229–231

17. Stocklin E, Wissler M, Gouilleux F, Groner B 1996 Functional interactions between Stat5 and the glucocorticoid receptor. *Nature* 383:726–728
18. Ruan W, Kleinberg DL 1999 Insulin-like growth factor I is essential for terminal end bud formation and ductal morphogenesis during mammary development. *Endocrinology* 140:5075–5081
19. Gual P, Baron V, Lequoy V, Van Obberghen 1998 Interaction of Janus kinases JAK-1 and JAK-2 with the insulin receptor and the insulin-like growth factor-1 receptor. *Endocrinology* 139:884–893
20. Hilfiker H, Hattenhauer O, Traebert M, Forster I, Murer H, Biber J 1998 Characterization of a murine type II sodium-phosphate cotransporter expressed in mammalian small intestine. *Proc Natl Acad Sci USA* 95:14564–14569
21. Groner B, Gouilleux F 1995 Prolactin-mediated gene activation in mammary epithelial cells. *Curr Opin Genet Dev* 1995 5:587–594
22. Ali S 2000 Recruitment of the protein-tyrosine phosphatase SHP-2 to the C-terminal tyrosine of the prolactin receptor and to the adaptor protein Gab2. *J Biol Chem* 275:39073–39080
23. Hang J, Rillema JA 1998 Possible involvement of P13K in prolactin-stimulated milk product formation and iodide transport in mouse mammary explants. *Proc Soc Exp Biol Med* 219:154–159
24. Vara JA, Caceres MA, Silva A, Martin-Perez J 2001 Src family kinases are required for prolactin induction of cell proliferation. *Mol Biol Cell* 12:2171–2183
25. Fresno Vara JA, Carretero MV, Geronimo H, Ballmer-Hofer K, Martin-Perez J 2000 Stimulation of c-Src by prolactin is independent of Jak2. *Biochem J* 345(Pt 1): 17–24
26. Das R, Vonderhaar BK 1996 Activation of raf-1, MEK, and MAP kinase in prolactin responsive mammary cells. *Breast Cancer Res Treat* 40:141–149
27. Robinson G, Accili D, Hennighausen L 2000 In: Ip M, Asch H, eds. *Methods in mammary gland biology and breast cancer research*. New York: Kluwer Academics/Plenum Publishers; 307–316
28. DeOme KB, Faulkin LJ, Bern J, Blair PE 1959 Development of mammary tumors from hyperplastic alveolar nodules transplanted into gland-free mammary fat pads of female C3H mice. *Cancer Res* 19:515–520
29. Kordon EC, McKnight RA, Jhappan C, Hennighausen L, Merlino G, Smith GH 1995 Ectopic TGF  $\beta$  1 expression in the secretory mammary epithelium induces early senescence of the epithelial stem cell population. *Dev Biol* 168:47–61
30. Seagroves TN, Lydon JP, Hovey RC, Vonderhaar BK, Rosen JM 2000 C/EBP $\beta$  (CCAAT/enhancer binding protein) controls cell fate determination during mammary gland development. *Mol Endocrinol* 14:359–368

